Procter&Gamble

The Procter & Gamble Company
Sharon Woods Technical Center
11511 Reed Hartman Highway, Cincinnati, Ohio 45241-9974

February 2, 2001

Dr. Scott Masten
NIEHS/NTP
P.O. Box 12233
Research Triangle Park
North Carolina, 27709

Dear Dr. Masten:

This recommends nomination of all-*trans*-retinyl palmitate for phototoxicity and photocarcinogencity testing be reevaluated and ultimately withdrawn. This recommendation, submitted in response to the December 4, 2000 *Federal Register* [Volume 65, Number 233, pg. 75727-75730] notice entitled "Request for comments on substances nominated to the national Toxicology Program (NTP) for toxicological studies and on the testing recommendations made by the NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC)", is based on the following rationale:

- All toxicological properties of retinyl palmitate, including any potential phototoxicity and photocarcinogenicity, are indistinguishable from the vitamin A family. Retinyl palmitate is formed reversibly in human skin and serves as an endogenous storage form of retinol (vitamin A). Retinyl palmitate is neither a synthetic retinoid nor a new chemical entity.
- Dietary retinyl palmitate has been reported to inhibit or have no effect on the formation of murine skin tumors produced by exposure to artificial ultraviolet (UV) radiation. Rodent photocarcinogenicity studies with other retinoids have produced equivocal results and, despite 25 years of work, remain controversial. As such, conduct of the proposed studies would provide no new scientific information beyond what is already known or suspected based on the rather extensive phototoxicological and photo co-carcinogenicity testing of provitamin A (i.e., β-carotene), vitamin A, and oxidative products (i.e., retinoic acid).
- The human relevance of rodent photocarcinogenicity or photo co-carcinogenicity testing is unknown. In contrast to carcinogenicity testing advanced and standardized by NTP over the past 23 years, rodent photo co-carcinogenicity testing has no *a priori* outcome criteria that classify results and aid in the interpretation of such studies. Rather, rodent photo co-carcinogenicity testing appears to be highly dependent on the testing (i.e., protocol) conditions. Most importantly, retinoids, natural or synthetic, are recognized by the medical community as emerging skin cancer treatment modalities.

Based on this rationale, it is our considered opinion that the proposed testing of all-*trans*-retinyl palmitate is unwarranted. The proposed NTP/NCTR studies would provide no new mechanistic understanding upon which regulatory or human risk assessment decisions could be based. Arguably, a regulatory decision for retinyl palmitate could be made without any additional testing. Moreover, the human relevance of such rodent photo co-carcinogenicity testing is unknown and, as such, would seem inconsistent with the respected and prestigious mission of the NTP. In an era of increasing fiscal and ethical responsibility, such testing represents a poor utilization of precious resources, namely money and laboratory animals.

The data supporting our recommendation that phototoxicity and photocarcinogenicity testing of all-*trans*-retinyl palmitate be withdrawn from consideration are presented in more detail in the following sections. There is, by design, some redundancies with the October, 2000 document entitled "All-*Trans*-Retinyl Palmitate [CASRN 79-81-2]" prepared by the Center for Food Safety and Applied Nutrition (CFSAN). As well, the scope of our comments are limited because the volume of scientific investigation of retinoids is enormous. Nonetheless, it is important to document the basis of our recommendation to forego phototoxicity and photocarcinogenicity testing of retinyl palmitate.

1. Relationship Among Retinoids

Vitamin A, retinol, is the prototype of all natural retinoids. Figure 1 provides the relationship among retinol, its storage form, i.e., retinyl palmitate, and oxidative products.

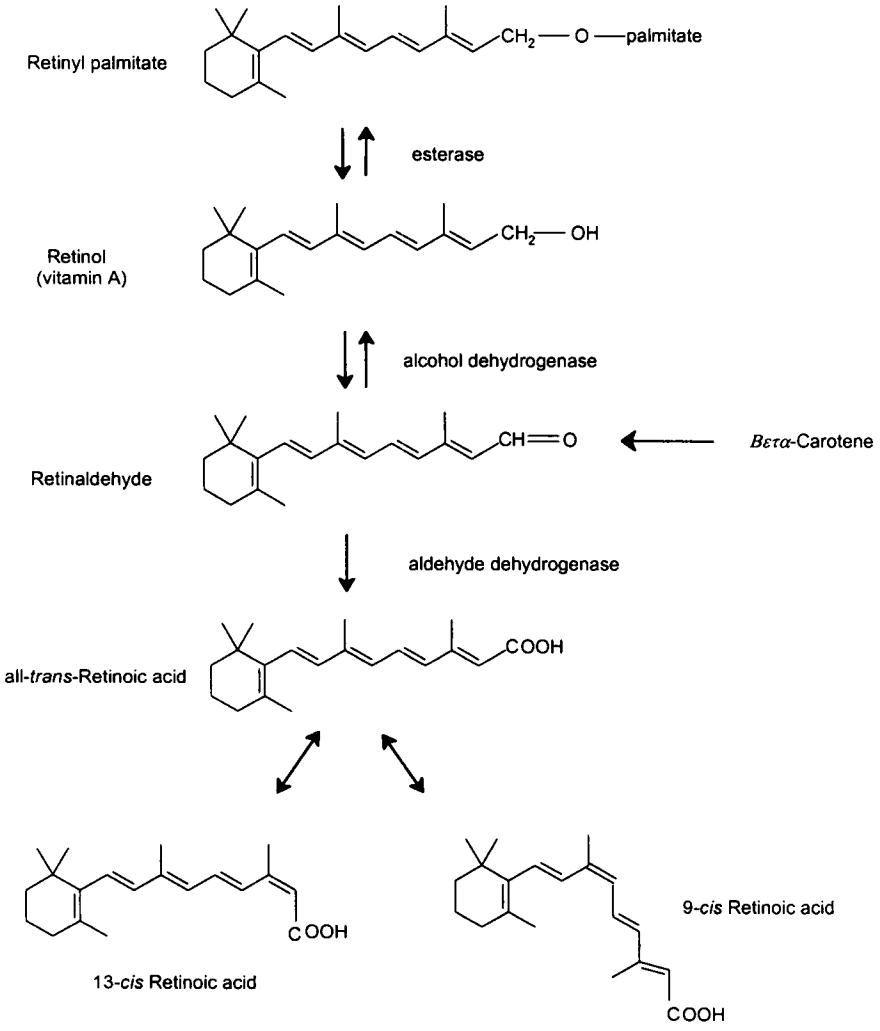


Figure1. Relationship between Vitamin A, retinol, its storage form and oxidative pathway

In most general terms, vitamin A from dietary sources, e.g., *Beta*-carotene and retinyl esters, is absorbed and transported in the blood as the bound form, i.e., retinol - retinol binding protein - transthyretin, to organs throughout the body. In tissue, retinol is actively taken up by cells and converted primarily to its

storage form, e.g., retinyl palmitate, and to a lesser extent, to the active hormone, all-*trans*-retinoic acid (Blomhoff et al. 1990) in a manner consistent with physiological need.

There is evidence that these pathways occur in the skin. Specifically, basal keratinocytes are supplied with vitamin A (i.e., retinol -retinol binding protein - transthyretin) from the bloodstream, and although the precise mechanism(s) is not completely understood, retinol gains entry into the cells through receptor-dependent and independent processes (Soprano and Blaner, 1994). Once inside the cell, retinol may be converted to retinyl palmitate or sequentially oxidized to retinoic acid. The reversible conversion of retinol to retinyl palmitate is believed to occur *via* retinyl esterase activities residing in a number of subcellular locations (Harrison, 1993; Harrison *et al.*, 1995) and through non-specific esterases which are abundant in the skin (McCraken *et al.*, 1993).

There can be no doubt that an intracellular mechanism for reversible conversion of retinol to retinyl palmitate exists in the skin. The formation of retinyl palmitate serves, in part, to control the availability of retinol. Specifically, in a squamous differentiating tissue like the skin, an elaborate vitamin A control network must exist in order to maintain proliferative and differentiative homeostasis. Since retinyl esters are the primary storage form for cellular vitamin A, the cell needs the machinery to mobilize these stores in times of need and, conversely, control the formation of oxidative products in times of excess. Therefore, although topical application of retinyl palmitate may be considered a non-physiological route of exposure, there is ample evidence to support the view that the skin possesses all of the enzymatic machinery necessary to convert retinyl palmitate to retinol (Boehnlein *et al.*, 1994; Fthenakis *et al.*, 1991; Thom, 1993). Accordingly, the small amount of retinyl palmitate that actually penetrates the skin would be expected and has been indirectly demonstrated to enter the normal physiological pathways controlling vitamin A homeostasis (Duell, *et al.* 1997).

The link between retinyl palmitate and retinol being clear, it follows that a small amount of retinol can be sequentially converted to retinaldehyde (Saurat *et al.*, 1995) and then to retinoic acid, the active form of vitamin A. In this regard, Kurlandsky *et al.* (1994) found that administration of retinol to cultured keratinocytes can activate a reporter gene linked to a retinoid response element. This event is dependent on the ligand, retinoic acid, binding to its receptor and activating transcription. Therefore, one can infer that retinoic acid was generated from retinol. If retinol oxidation is inhibited, there is no activation of this reporter gene. Also, if retinol is applied to the skin *in vivo*, increases in expression of genes which contain retinoid response elements in their regulatory regions (i.e. CRABP and CRBP) occurs (Kang et al., 1995). Thus, retinyl palmitate is linked to the production of retinol and ultimately retinoic acid which presumably mediates any effect of the ester.

Given the pathway presented in Figure 1 and the preceding discussion, it should be clear that the pharmacological/toxicological differences between retinyl palmitate, retinol and retinoic acid are strictly quantitative (for review see Biesalsky, 1989). Thus, responsiveness to vitamin A and its derivatives can be rank ordered based on the production of retinoic acid and its binding to nuclear hormone receptors (Darmon *et al.*, 1988). These receptors, RARs and RXRs, are known to mediate many, if not all of the differentiation-associated effects of vitamin A in the epidermis (for review see Roos *et al.*, 1998). In this regard, topical application of retinyl palmitate to human (Thom, 1993; Fthenakis *et al.*, 1991) or animal (Counts et al., 1988) skin produces retinoid effects at much lower potency.

The biochemical interdependence between retinyl palmitate, retinol and ultimately retinoic acid has led to the suggestion that retinoic acid is sufficient to serve all functions of vitamin A in epithelial tissues. In this regard, there is biological evidence that retinoic acid can correct the epithelial abnormalities that attend vitamin A deficiency (Jetten, 1991). As well, at much higher concentrations, retinyl esters including retinyl palmitate can correct the physiological perturbations arising from vitamin A deficiency.

Having established the relationship between retinyl palmitate and retinol/retinoic acid, it would seem illogical and with indifference to scientific principles to suggest that retinyl palmitate would possess novel pharmacological and toxicological properties, including phototoxicity or photocarcinogenicity, beyond those of retinol. This essential relationship allows a singular extrapolation of studies conducted with retinyl palmitate, retinol, retinal and retinoic acid accepting only a quantitative difference in response.

2. Phototoxicological Studies with Retinyl Palmitate and other Retinol Derivatives

There is an obvious and undeniable controversy regarding the phototoxicological and photocarcinogenic effects of retinoids. Specifically, since 1977 there have been many studies conducted in rodents which have found dietary/oral or topical exposure to retinoids to enhance, have no effect, or prevent the appearance of skin tumors produced by a variety of artificial UV sources. The divergent beliefs have been presented in various reviews (Kligman, 1987; Davies and Forbes, 1988; Kligman, 1993) and presented in the October, 2000 CFSAN document The existence of such conflicting results may suggest that additional studies are warranted. However, it is our view that additional data regardless of the degree of scientific rigor, including the presumed relevant artificial light source or conditions relevant to use of retinyl palmitate in cosmetics, are unlikely to resolve the existing controversy or advance regulatory or human health decision making. In fact, any outcome would only add to the controversy.

It is, however, worthwhile examining some of the work in this area including that presented in the October 2000 CFSAN document. Again, it should be noted that given the experimental complexity and the immense database it is not possible to consider all studies or their methodological details but rather those which have direct bearing on the nomination of all-*trans*-retinyl palmitate for phototoxicity and photocarcinogenicity testing.

2.1 Rodent Photocarcinogenicity Studies with Retinyl Palmitate

The effect of a dietary retinoid, retinyl palmitate, on UV-induced immunosuppression and skin tumorigenesis has been investigated by Gensler and colleagues (Gensler, 1989; Gensler and Holladay, 1990; Gensler *et al.*, 1990). In these studies, female C3H/HeN mice were fed a diet containing 120 IU retinyl palmitate/g food for 18 weeks to allow significant cutaneous accumulation of retinol and retinyl palmitate (Peng *et al.*, 1986). The source of UV in these studies consisted of unfiltered FS-40 fluorescent sun lamps the spectral output of which is 280-340 nm. In the study of Gensler *et al.* (1990) the tumor burden, i.e., number of tumors/mouse, but not tumor incidence was significantly reduced by the diet enriched with retinyl palmitate.

The study of Kelly *et al.* (1989) confirm the findings of Gensler and colleagues. Kelly *et al.* administered Vitamin A palmitate, 60 and 300 IU of vitamin A, by gavage, three times a week to SKH1 albino hairless mice. The animals were exposed to UV, daily Monday to Friday, for 25 weeks. The artificial source of UV was from a bank of 6 fluorescent 40 watt tubes comprised of one UVB (FL40SE), three UVA (F40/350 BL) and two True-Lite tubes. The vitamin A palmitate had no effect on time of onset of tumors, number or types of tumors compared to vehicle-treated control mice.

These independent sets of data in two different strains of mice fed a diet containing retinyl palmitate, which increased skin concentrations of retinol and retinyl palmitate in the target tissue, reported no effect on UV-induced skin tumor formation under the conditions of these studies. It is difficult to imagine how topical retinyl palmitate might produce a qualitatively different outcome considering the documented increase in concentrations of retinol and retinyl palmitate in the skin. This view is supported by the therapeutic response to oral and topical retinoids in acne patients.

2.2 Rodent Photocarcinogenicity Studies with Beta-Carotene (Provitamin A)

Beta-carotene has long been of interest as a potential chemopreventive agent due to its antioxidant properties and the potential benefit such properties hold in prevent/reducing tumorigenesis (for review see Black and Mathews-Roth, 1991). As such, animal studies have been conducted to determine the

ability of beta-carotene to prevent UV-induced skin tumorigenesis. Epstein (1977) first demonstrated that beta-carotene administered intraperitoneally to albino hairless mice reduced the size and increased the latency for tumor formation in response to UV emitted from a Hanovia hot quartz lamp. These results were replicated in a study by Mathews-Roth (1982) using SKH1 hairless mice administered beta-carotene in the diet and exposed to UV from unfiltered FS-20 sunlamps. However, in these studies, the protective effect of beta-carotene was not related to its vitamin A activity.

Black and Mathews-Roth (1991) suggest that the interest in the ability of beta-carotene to prevent cancer was heightened by the review of Peto *et al* (1981). At that time (1991), a total of 12 intervention trials sponsored by the National Institutes of Health were in progress to evaluate the potential for beta-carotene to prevent different cancers in high risk individuals. Obviously, work continues to progress investigating the potential benefits of beta-carotene. Whereas the antioxidant properties of beta-carotene may be responsible for any benefit, based on its conversion to retinal, the vitamin A pathway cannot be excluded as a possible contributory mechanism.

Regardless, the limited photocarcinogenicity findings with beta-carotene suggest a protective effect against UV-induced skin tumorigenesis.

2.3 Rodent Photocarcinogenicity Studies with Retinol (Vitamin A)

In a carefully conducted study by Mikkelsen *et al.* (1998) lightly pigmented hairless mice were fed a laboratory diet enriched with vitamin A at two doses which resulted in a 4 fold difference in the concentration in the skin. After the mice were maintained for 4 weeks on the diets, daily irradiation with UVB FS 40 sunlamp or UVB + some UVA from Philips TL 40) were begun for 18 weeks. The tumor incidence was significantly greater in the animals maintained on the vitamin A enriched diet compared to those administered the control diet. The authors suggest that the excessive vitamin A may decompose and generate short-lived free radicals in the skin that may enhance UV-induced skin tumorigenesis.

This study would suggest that under the conditions of this study, vitamin A enhances UV-induced skin tumorigenesis.

2.4 Rodent Photocarcinogenicity Studies with Retinoic Acid

These are the most controversial of the retinoid data with respect to photocarcinogenesis. The results obtained over 25 years demonstrate the indeterminate outcome of such studies. As presented in the reviews by Kligman (1987; 1993) and Davies and Forbes (1988) there are thoughtful explanations for the disparate results. Whereas much can be learned by comparing and contrasting such studies, it ultimately leads to the more pressing question: How do these data relate to humans?

Prior to considering this important question, a brief summary of the results with retinoic acid will be discussed. Table 1 summarizes results from studies examining the effect of topical retinoic acid on UV-induced skin tumorigenesis.

There are, as well, numerous studies submitted to the Food and Drug Administration (FDA) examining the effects of retinoids and structural analogs on UV-induced skin tumorigenesis. Some of these have been presented in the review by Jacobs *et al.*, (1999). As is the pattern with these studies, even when the experimental parameters, i.e., light source, strain of mouse, pattern of treatment, etc., are controlled for, the results are mixed.

Table 1. Summary of Studies Investigating the Effect of Topical Application of Retinoic Acid on UV-Induced Skin Tumorigenesis in Rodent

Study	Effect of topical retinoic acid on UV-induced carcinogenesis
Epstein, 1977	enhancement
Forbes *et al.*, 1979	enhancement
Epstein and Grekin, 1981	inhibition
Forbes, 1981	enhancement
Hartmann and Teeelman, 1981	enhancement
Kligman and Kligman, 1981	no enhancement
Connor *et al.*, 1983	inhibition
Davies and Forbes, 1988	enhancement
Kligman and Crosby, 1996	inhibition
Halliday et al., 2000	enhancement

Because retinoic acid is the most widely studied retinoid and, assuredly the active form of vitamin A and retinyl palmitate in epithelia tissue, it is noteworthy that the results are conflicting. This ambiguity is reflected in the warning statement for Retin A® (all-*trans*-retinoic acid:

- Studies in hairless albino mice suggest that tretinoin may enhance the tumorigenic potential of ultraviolet (UV) light from a solar simulator. In other studies, when lightly pigmented hairless mice treated with tretinoin were exposed to carcinogenic doses of UVA/UVB light, the incidence and rate of development of skin tumors were either reduced or no effect was seen. Due to significantly different experimental conditions, no strict comparison of these disparate data is possible. **Although the significance of these studies to humans is not clear, patients should avoid or minimize exposure to sun**.

Considering the totality of these data, it is our view that the proposal for the NCTR Phototoxicology Center to conduct phototoxicity and photocarcinogenicity testing of all-*trans*-retinyl palmitate will simply add more information without addressing the most important question "How do these photo co-carcinogenicity data relate to human risk?"

3. Human Relevance of Rodent Photo co-carcinogenicity Testing: The Example of Retinoids

It is acknowledged that the predictivity of rodent photocarcinogenicity or photo co-carcinogenicity testing for humans is unknown and thus the relevance of the rodent studies for human health risk cannot be reliably assessed. This is not for lack of trying, but rather that the human examples upon which any predictive toxicological test must be based are limited to one example (i.e., 8-methoxypsorlen + UVA or PUVA therapy for psoriasis). Provided at least part of the mission of the NTP is to provide hazard data from which human risk assessment may be considered, the uncertainty associated with the outcome of the rodent photo co-carcinogenicity test would not help fulfill such an obligation.

No example is more germane to this issue than that of retinoids. In considering the value of conducting a rodent photo co-carcinogenicity study with retinyl palmitate, it is worthwhile reviewing the human evidence. In this regard, the *a priori* hypothesis based on existing data and proposed testing would be that exposure to retinoids, i.e., retinyl palmitate, retinol, retinoic acid, enhances skin tumorigenesis produced by exposure to solar UV.

Aside from the many experimental concerns, anecdotal reports and incomplete accounts of retinoids, it is clear that the *a priori* hypothesis has little practical basis and is diametrically opposed to the collective medical experience in humans.

3.1 Relevant Human Cancer Studies

There are two extremely important observations in humans which have direct bearing on the question of exposure to vitamin A and its derivatives and enhancement of UV-induced skin carcinogenesis. These are the condition of xeroderma pigmentosum and organ transplant recipients, both conditions dramatically increasing the risk of UV skin carcinogenesis many times.

In the study of Kraemer *et al* (1988) a group of 5 xeroderma pigmentosum patients were surgically rid of all pre-existing tumors (BCC, SCC), then treated with high dose oral 13-cis retinoic acid (see Figure 1) for two years, and were followed for 1 year off the drug. During treatment there was an average reduction in skin cancers of 63%; following cessation of treatment, there was an 8.5-fold increase in tumor frequency. All patients experienced side effects of this treatment. Nonetheless, in this exquisitely sensitive population, oral retinoic acid had a clinically beneficial effect.

The incidence of skin cancer in organ transplant recipients is extraordinarily high. The degree of immunosuppression independent of the pharmacotherapy is believed to be responsible for the increased risk of skin cancer in these patients (Bouwes Bavinck *et al.*, 1996). In the report by McKenna and Murphy (1999) the effect of acitretin was evaluated in 16 patients over a 5 year period. There was a significant reduction in the number of new tumors in these subjects, demonstrated over a 4 years of treatment. Thus, like xeroderma pigmentosum, these subjects benefit from treatment with retionids.

<u>These two examples are diametrically opposed to studies showing enhancement of UV-induced skin tumorigenesis in mice.</u>

Aside from the two examples, much work has been done investigating chemopreventive properties of retinoids (for review see Craven and Griffiths, 1996; Lotan 1996). The prevailing view of the medical community is that retinoids provide a benefit in the treatment of cancers.

Perhaps, the most important point of the preceding discussion is that there does not appear to exist prospective clinical studies, drug trials or anecdotal reports which would support the hypothesis that exposure to retinoids enhances UV-induced skin tumorigenesis. This statement is, of course, subject to much debate and skepticism. Nonetheless, the forces of clinical medicine for the past 25 years have been either unaware of the potential concerns of the retinoids or of the mind that such risks are manageable (i.e., avoid sunlight the primary cause of human skin cancers).

4. Conclusion:

In the review entitled "Comparative assessment of the toxicology of Vitamin A and retinoids in man", the author, Hans Biesalski, makes the following observations:

- "It is almost impossible to make a genuine distinction between toxic symptoms induced by retinyl esters and retinol".
- " . . . there is little point in describing typical side-effects of retinyl esters in isolation"
- "A better distinction is possible with the acid derivatives of the vitamins, the retinoids."

These points are directly applicable to the nomination of retinyl palmitate for photo co-carcinogenicity testing. First, the relationship between retinyl palmitate, retinol, retinal and retinoic acid is incontrovertible. To suggest that retinyl palmitate possess pharmacological and toxicological properties outside of those already characterized for vitamin A is incompatible with the biochemical and physiological relationship among these retinoids. Second, the results of several photocarcinogenicity or photo co-carcinogenicity studies suggest that depending on the conditions of the study, UV-induced tumorigenesis may be enhanced, prevented or not affected by retinyl palmitate, retinol or retinoic acid. Third, rodent photo co-carcinogenicity testing is akin to tumor promotion. With the current state of

knowledge it is not possible to extrapolate results from mouse tumor promotion studies to humans with any degree of certainty. For instance, different stocks and strains of mice vary substantially in their responsiveness to skin tumor promoters and the bases for this variation are unknown. Therefore, the human relevance of data obtained in the responsive strains is unclear. In general, rodent skin tumor promotion effects show substantial strain and species specificity, which also makes inter-species extrapolation uncertain. While much is known about the cellular effects of tumor promoters, the conditions necessary and sufficient for tumor promotion have not been established. Furthermore, it is not known whether tumor promotion as demonstrated in certain animal models occurs in human skin, and no human skin tumor promoters have been identified.

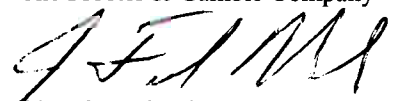
Equally important is the interpretation of any photo co-carcinogenicity study. Whereas the National Toxicology Program may have little or no interest in the application of results obtained from such studies, it is reasonable to suggest that the consequences be considered before valuable resources are devoted to such investigations. To reiterate, the testing proposed is not a carcinogenicity study in which detailed criteria exist for interpreting the outcome of the study. In fact, there are no criteria for considering a potential photo co-carcinogen. This is likely the result of a lack of understanding regarding such an outcome. For retinoids this is obvious. Retinoic acid either enhances, has no effect or inhibits UV-induced skin tumor formation in animals. In stark contrast, the clinical use of retinoic acid to treat the very lesion it has been suggested they produce continues.

We strongly believe that the conduct of photo co-carcinogenicity testing of retinyl palmitate is unwarranted based on the following:

- the equivocal results from rodent photo co-carcinogenicity studies of retinoids,
- the absence of an adverse effect in prospective human studies and the use of retinoids to treat skin cancers in subjects with xeroderma pigmentosum, patients with systemic immunosuppression to prevent organ rejection, and for treating actinic keratosis and photodamage; and
- the uncertainty associated with the outcome of SKH1 albino hairless mouse photo co-carcinogenicity with regard to criteria for interpretation and, most important, human risk.

Many would consider the testing of retinyl palmitate an unnecessary use of laboratory animals and waste of valuable resources. We recommend the phototoxicity and photocarcinogenicity testing of retinyl palmitate not be conducted for the reasons detailed in this submission.

Respectfully
The Procter & Gamble Company



J Frank Nash, Ph.D.
Principal Scientist

4. References

Biesalski, H.K.. Comparative assessment of the toxicology of vitamin A and retinoids in man. *Toxicology*, **57**: 117-161, 1989.

Black, H.S. and Mathews-Roth, M.M. Protective role of butylated hydroxytoluene and certain carotenoids in photocarcinogenesis. *Photochem. Photobiol.* **53**(5): 707-716, 1991.

Blomhoff, R., Green, M.H., Berg, T. and Norum, K.R. Transport and storage of vitamin A. *Science*, **250**: 399-404, 1990.

Boehnlein, J., Sakr, A., Lichtin, J.L. and Bronaugh, R.L. Characterization of esterase and alcohol dehydrogenase activity in skin. Metabolism of retinyl palmitate to retinol (vitamin A) during percutaneous absorption. *Pharm. Res.*, **11**(8): 1155-1159, 1994.

Bouwes Bavinck, J.N., Hardie, D.R., Green, A., Cutmore, S., MacNaught, A., O'Sullivan, B., Siskind, V., vanderWoude, F.J. and Hardie, I.R. The risk of skin cancer in renal transplant recipients in Queensland, Australia. *Transplantation,* **61**(5): 715-721, 1996.

Connor, M.J., Lowe, N.J., Breeding, J.H. and Chalet, M. Inhibition of ultraviolet-B skin carcinogenesis by all-*trans*-retinoic acid regimens that inhibit ornithine decarboxylase induction. *Cancer Res.* **43**: 171-174, 1983.

Counts, D.F., Skreko, F., McBee, J. and Wich, A.G. The effect of retinyl palmitate on skin composition and morphometry. *J. Soc. Cosmet.Chem.*, **39**: 235-240, 1988.

Darmon, M., Rocher, M., Cavey, M.-T., Martin, B., Railloud, T., Delescluse, C., Shroot, B. Biological activity of retinoids correlates with affinity for nuclear receptors but not for cytosolic binding proteins. *Skin Pharmacol.* **1**: 161-175, 1988.

Davies, R.E. and Forbes, P.D. Retinoids and photocarcinogenesis: a review. *J. Toxicol. - Cut. & Ocular Toxicol.* **7**(4): 241-253, 1988.

Duell, E.A., Kang, S., and Voorhees, J.J. Unoccluded retinol penetrates human skin *in vivo* more effectively than unoccluded retinyl palmitate or retinoic acid. *J. Invest. Dermatol.* **109**: 301-305.

Epstein, J.H. Effects of β-carotene on ultraviolet induced cancer formation in the hairless mouse skin. *Photochem. Photobiol.*, **25**: 211-213, 1977.

Epstein, J.H. Chemicals and photocarcinogenesis. *Aust. J. Dermatol.* **18**: 57-61.

Epstein, J.H. and Grekin, D.A. Inhibition of ultraviolet-induced carcinogenesis by all-trans retinoic acid. *J Invest. Dermatol.*, **76**(3): 178-180, 1981.

Forbes, P.D. Photocarcinogenesis: an overview. *J Invest. Dermatol.* **77**: 139-143.

Forbes, P.D., Urbach, F. and Davies, R.E. Enhancement of experimental photocarcinogenesis by topical retinoic acid. *Cancer Letters*, **7**: 85-90, 1979.

Frieling, U.M., Schaumberg, D.A., Kupper, T.S., Muntwyler, J. and Hennekens, C.H. A randomized, 12-year primary-prevention trial of beta carotene supplementation for nonmelanoma skin cancer in the physicians' health study. *Arch. Dermatol.*, **136**: 179-184, 2000.

Fthenakis, C.G., Maes, D.H. and Smith, W.P. *In vivo* assessment of skin elasticity using ballistometry. *J. Soc. Cosmet. Chem.*, **42**: 211-222, 1991.

Gensler, H.L. Reduction of immunosuppression in UV-irradiated mice by dietary retinyl palmitate plus canthaxanthin. *Carcinogenesis*, **10**(1): 203-207, 1989.

Gensler, H.L, Aickin, M., and Peng, Y.M. Cumulative reduction of primary skin tumor growth in UV-irradiated mice by the combination of retinyl palmitate and canthaxanthin. *Cancer Lett.*, **53**(1): 27-31, 1990.

Gensler, H.L. and Holladay, K. Enhanced resistance to an antigenic tumor in immunosuppressed mice by dietary retinyl palmitate plus canthaxanthin. *Cancer Lett.* **49**(3): 231-236, 1990.

Halliday, G.M., Robertson, B.O. and Barnetson, R.C. Topical retinoic acid enhances, and a dark tan protects, from subedemal solar-simulated photocarcinogenesis. *J. Invest. Dermatol.*, **114**, 923-927, 2000.

Harrison, E.H. Enzymes catalyzing the hydrolysis of retinyl esters. *Biochim. Boiphys. Acta*, **1170** (2): 99-108, 1993.

Harrison, E.H., Gad, M.Z. and Ross, C.A. Hepatic uptake and metabolism of chylomicron retinyl esters: probable role of plasma membrane/endosomal retinyl ester hydrolases. *J. Lipid Res.*, **36**: 1498-1506, 1995.

Hartmann, H.R. and Teelmann, K. The influence of topical and oral retinoid treatment on photocarcinogenicity in hairless albino mice. In: Retinoids: Advances in Basic Research and Therapy, eds. Orfanos, C.E., Braun-Falco, O., Farber, E.M., Gr;upper, Ch., Polano, M.K. and Schuppli, R. Springer-Verlag, Berlin, pgs. 447-458.

Jacobs, A., Avalos, J., Brown, P. and Wilkin, J. Does photosensitivity predict photococarcinogenicity? *International J. Toxicol.* **18**: 191-198, 1999.

Jetten, A.M. Growth and differentiation factors in tracheobronchial epithelium. *Am. J. Physiol.*, **260**: L361-L373, 1991.

Kang, S., Duel, E.A., Fisher, G.J., Datta, S.C., Wang, Z-Q, Reddy, A.P., Tavakkol, A., Yi, J.Y., Griffiths, C.E.M., Elder, J.T. and Voorhees, J.J. Application of retinol to human skin *in vivo* induces epidermal hyperplasia and cellular retinoid binding proteins characteristic of retinoic acid but without measurable retinoic acid levels or irritation. *J. Invest. Dermatol.*, **105**: 549-556, 1995.

Kelly, G.E., Meikle, W.D. and Sheil A.G.R. Effects of oral retinoid (vitamin A and etretinate) therapy on photocarcinogenesis in hairless mice. *Photochem. Photobiol.* **50**(2): 213-215, 1989.

Kligman, L.H. Retinoic acid and photocarcinogenesis - a controversy. *Photodermatol.* **4**: 88-101, 1987.

Kligman, L.H. Tretinoin and photocarcinogenicity: an update. *J. Toxicol. - Cut. & Ocular Toxicol.* **12**(3): 205-220, 1993.

Kligman, L.H. and Crosby, M.J. Topical tretinoin enhances corticosteroid-induced inhibition of tumorigenesis in hairless mice previously exposed to solar simulating radiation. *Cancer Letters*, **107**: 217-222, 1996.

Kligman L.H. and Kligman, A.M. Lack of enhancement of experimental photocarcinogenesis by topical retinoic acid. *Arch. Dermatol. Res.*, **270**: 453-462, 1981.

Kraemer, K.H., DiGiovanna, J.J., Moshell, A.N., Tarone, R.E. and Peck, G.L. Prevention of skin cancer in xeroderma pigmentosum with the use of oral isotretinoin. *N. Engl. J. Med.*, **318**(25): 1633-1637, 1988.

Kurlandsky, S., Xiao, J-H., Duell, E.A., Voorhees, J.J.and Fisher, G.J. Biological activity of all-*trans* retinol requires metabolic conversion to all-*trans* retinoic acid and is mediated through activation of nuclear retinoid receptors in human keratinocytes. *J. Biol. Chem.*, **269**: 32821-32827, 1994.

Lotan, R. Retinoids in cancer chemoprevention. *FASEB J.*, **10**: 1031-1039, 1996.

Mathews-Roth, M.M. Antitumor activity of Beta-corotene, canthaxanthin, and phytoene. *Oncology* **39**, 33-37, 1982.

McCracken, N.W., Blain, P.G. and Williams F.M. Nature and role of xenobiotic-metabolizing esterases in rat liver, lung, skin and blood. *Biochem. Pharmacol.*, **45**: 31-36, 1993.

McKenna, D.B. and Murphy, G.M. Skin cancer chemoprophylaxis in renal transplant recipients: 5 years of experience using low-dose acitretin. *Br. J. Dermatol.*, **140**: 656-660, 1999.

Mikkelsen, S., Berne, B., Staberg, B. and Vahlquist, A. Potentiating effect of dietary vitamin A on photocarcinogenesis in hairless mice. *Carcinogenesis* **19**(4) 663-666, 1998.

Peng, Y.M., Alberts, D.S., Xu, M.J., Watson, R.R., Gensler, H.L. and Bowden, G.T. Effects of high dietary retinyl palmitate and selenium on tissue distribution of retinoids in mice exposed to tumor initiation and promotion. *J. Nutr. Growth and Cancer* **3**: 38-45, 1986.

Peto, R., Doll, R., Buckley, J.D. and Sporn, M.B. Can dietary β-carotene materially reduce human cancer rates? *Nature* **290**: 201-208, 1981

Roos, T.C., Jugert, F.K., Merk, H.F. and Bickers, D.R. Retinoid metabolism in the skin. *Pharmacol. Rev.* **50**: 315-333, 1998.

Saurat, J.H., Didierjean, L., Masgrau, E., Piletta, P.A., Jaconi, S., Chatellard-Gruaz, D., Gumowski, D., Masouye, I., Saloman, D. and Siegenthaler, G. Topical retinaldehyde on human skin: biologic effects and tolerance. *J. Invest. Dermatol.*, **103**: 770-774, 1995.

Soprano, D.R. and Blaner, W.S. Plasma retinol binding protein. In, *The Retinoids: Biology, Chemistry, and Medicine*, 2 ed., Eds. Sporn, M.B., Roberts, A.B., and Goodman, D.S., Raven Press, Ltd., New York, 1994.

Thom, E. Skin treatment with two different Galenical formulations of retinyl palmitate in humans. *J. Appl. Cosmetol.*, **11**:71-76, 1993.

Torma, H. and Vahlquist, A. Vitamin A esterification in human epidermis: a relation to keratinocyte differentiation. *J Invest Dermatol.* **94**(1): 132-138, 1990.